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Research Article

The effect of essential oils of thyme, peppermint, savory and two fungicides on the growth of three plant pathogenic fungi

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Abstract

Introduction: Some plant essential oils have antifungal properties. This study was conducted to determine the effect of three plant essential oils and two chemical fungicides on the growth of three plant pathogenic fungi in vitro. **Materials and methods:** The effect of different concentrations of three essential oils of thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperata* L.) and khuzestani savory (*Satureja khuzestanica* Jamzad), compared to two chemical fungicides; mancozeb and carbendazim were investigated on the growth of three fungi *Alternaria solani*, *Botrytis cinerea* and *Fusarium solani* by dilution in Yeast Extract Sucrose Broth medium method and their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were calculated. **Results:** Thyme essential oil had the greatest inhibitory effect on *A. solani* and *F. solani* among these essential oils, and savory essential oil had the greatest inhibitory effect on *B. cinerea*. Among the chemical fungicides, mancozeb had the most inhibitory effect on *A. solani* and carbendazim had the most inhibitory effect on *B. cinerea* and *F. solani*. **Conclusion:** Thyme and khuzestani savory essential oils have a significant inhibitory effect on the growth of these three plants pathogenic fungi.

Keywords: *Alternaria*, *Botrytis, Fusarium*, Carbendazim, Mancozeb

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مقاله پژوهشی

اثر اسانسهای آویشن، نعناع فلفلی، مرزه و دو قارچ‌کش بر رشد سه قارچ بیماری‌زای گیاهی

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چکیده

مقدمه: بعضی اسانس‌های گیاهی خاصیت ضد قارچی دارند. این پژوهش برای تعیین اثر سه اسانس گیاهی و دو قارچکش شیمیایی بر رشد پرگنه سه قارچ بیمارگر گیاهی در شرایط آزمایشگاهی انجام شد. **مواد و روش‌ها:** اثر غلظت‌های مختلف سه اسانس آویشن (*Thymus vulgaris* L.)، نعناع فلفلی (*Mentha piperata* L.) و مرزه خوزستانی (*Satureja khuzestanica* Jamzad)، در مقایسه با دو قارچ‌کش مانکوزب و کاربندازیم، بر رشد سه قارچ *Alternaria solani*، *Botrytis cinerea* و *Fusarium solani* با روش رقیق‌سازی در محیط عصاره مخمر سوکرز مایع مورد بررسی قرار گرفتند و حداقل غلظت بازدارندگی (MIC) و حداقل غلظت قارچ‌کشی (MFC) آنها محاسبه شدند. **یافته‌ها:** اسانس آویشن در بین اسانهای گیاهی بیشترین اثر بازدارندگی را بر *A. solani* و *F. solani* و اسانس مرزه بیشترین اثر بازدارندگی را بر *B. cinerea* داشتند. مانکوزب در بین قارچکشهای شیمیایی بیشترین اثر بازدارندگی را بر *A. solani* و کاربندازیم بیشترین اثر بازدارندگی را بر *B. cinerea* و *F. solani* داشتند. **نتیجه‌گیری:** اسانس‌های آویشن و مرزه اثر بازدارندگی قابل ملاحظه‌ای بر رشد پرگنه این سه قارچ بیمارگر گیاهان دارند.

واژگان کلیدی: *Alternaria*, *Botrytis*, *Fusarium*, Carbendazim, Mancozeb

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Introduction مقدمه

Postharvest damage of fruits is significant, so that about 25% of the harvested fruits are damaged by pathogens during the harvesting, transportation and storage processes (Sharma et al. 2009). *Botrytis cinerea* Pers. Fr., the agent of gray mold disease in many garden products, is the most common pathogen causing storage rot in most regions of the world, which causes great damage even at low storage temperatures (Droby and Lichter 2004). The most important potato disease during storage is dry rot, caused mainly by *Fusarium solani* (Mart) Sacc. (Pringle et al. 2009). Moreover, *Alternaria solani* (Ell. & Mart.) Jones and Grout, is a saprophytic pathogenic fungus causes early blight disease in tomatoes, quickly after harvest (Wang et al. 2009). Indiscriminate use of chemical compounds, in addition to polluting the environment, causes the phenomenon of resistance to pesticides (Habbadi et al. 2017).

Nowadays, the antifungal effects of plant compounds have been proven on a large number of plant pathogens, and about 60% of these substances have the ability to inhibit the growth of fungi (Kordali et al. 2016). For example, aromatic plants belonging to the mint family (Lamiaceae) are rich in antimicrobial and antioxidant compounds, and the most important compounds of these plants are carvacrol and thymol. Thyme (*Thymus vulgaris* L.) is an aromatic plant from the mint family, whose antioxidant and antifungal properties have been proven (Gulluce et al., 2007). Peppermint (*Mentha piperita* L.) is one of the most important and widely used types of mint in traditional medicine, which has antimicrobial and antioxidant properties (Dhifi et al. 2013). Savory (*Satureja khuzestanica* Jamzad) is one of the plants that is not only widely used in traditional medicine due to its medicinal properties, but also its antimicrobial activity has been proven on some fungal isolates, due to its phenolic compounds, thymol and carvacrol, (Skocibusic et al. 2006). In recent years, many studies have been conducted on the effect of plant essential oils on plant pathogenic fungi, and the antifungal effects of some of these essential oils have been proven on *B. cinerea* (Banani et al. 2018). Antifungal effects of peppermint essential oil have also been shown at *F. solani* (Bang 2007). Also, the control of *A. solani* by savory essential oil has been indicated in the culture medium and tomato fruit tissue (Sesan et al. 2016). In the present study, the inhibitory effect of the essential oils of three species of medicinal plants: thyme, peppermint, and savory was investigated on the mycelium growth of three species of plant pathogenic fungi, *A. solani*, *B. cinerea* and *F. solani* in comparison with the effects of two fungicides: Mancozeb and Carbendazim.

Material and Methods مواد و روش‌ها

Preparation of fungal isolates

Botrytis cinerea, *Fusarium solani*, and *Alternaria solani* fungi were collected and isolated from infected kiwi, potato, and tomato fruits, respectively, from Khorramabad fruit and

vegetable market in 2017. After purification, identification of species were done based on various criteria such as presence or absence of cell wall, shape and size of conidiophore, conidia and phialides, number and color of conidia placed on conidiophore or phialides, single or multi-celled conidia, the diameter growth of the colony, the color and state of the colony, etc. (Barnett and Hunter 1995, Leslie and Summerell 2006).

Preparation of medicinal plants

Thyme, peppermint and savory plants were obtained from the medicinal plant farm of Golkaran Kesht and Sanaat, Kashan city.

Extracting essential oils from plants

The essential oil of the plant samples was obtained through hydro-distillation using Clevenger apparatus, after drying the aerial parts of the plants in the shade (Adams 1995).

Determination of minimum inhibitory concentration and minimum fungicidal concentration

To determine the minimum concentration of inhibition (MIC) and the minimum concentration of fungicide (MFC), the dilution method was used in the liquid culture medium in the broth macrodilution test tube (Griffein et al. 2000). First, Yeast Extract Sucrose Broth (YESB) culture medium was prepared and five milliliters of it was poured into each test tube. Then concentrations of 0 (sterile distilled water), 12.5, 25, 50, 200, 500, 1000, 1250 and 1500 microliters of each plant essential oils in Tween 20 solvents were added to the tubes. To determine the MIC and MFC of fungicides, 0 (sterol distilled water), 10, 20, 30, 40 and 50 micrograms of Mancozeb and Carbendazim fungicides were added to the tubes and like the essential oil method, Tween 20 was added (Zhang et al. 2006). The lowest concentration in which significant growth of the fungus was observed compared to the control, was considered as the minimum inhibitory concentration (Hellio et al. 2000). Then, the culture medium containing the fungus which did not grow in the essential oil treatment, was transferred to the PDA medium free of essential oil and fungicide to determine the reversibility of the growth inhibition, and if the fungal growth was irreversible after 10 days, the corresponding concentration was considered as the minimum fungicidal concentration (Galgiani et al. 1992).

Results **یافته‌ها**

Calculation minimum inhibitory concentrations, showed that the lowest MIC value belonged to the concentration of 12.5 µl/l of thyme essential oil on *F. solani* and the concentration of 12.5 µl/l of savory essential oil on *B. cinerea*. Also, the lowest MFC value was related to 500 µl/l savory essential oil on *B. cinerea* and *F. solani* fungi. The lowest MIC of the fungicides was related to 10 µl/l of mancozeb fungicide on *A. solani* and 10 µl/l of carbendazim on *B. cinerea* and *F. solani*. The results of the investigation of the minimum

جدول ۱. حداقل غلظت بازدارندگی (MIC) و حداقل غلظت قارچ‌کشی (MFC) سه اسانس گیاهی آویشن (Thyme)، نعناع فلفلی (Peppermint) و مرزه خوزستانی (Khuzestani savory) و دو قارچ‌کش شیمیایی مانکوزب و کاربندازیم، بر رشد *Alternaria solani*، *Botrytis cinerea* و *Fusarium solani* در شرایط آزمایشگاهی.

Table 1. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of three plant essential oils of thyme, peppermint and Khuzestani savory, and two chemical fungicides mancozeb and carbendazim, on the growth of *Alternaria solani*, *Botrytis cinerea* and *Fusarium solani* in vitro.

Treatment	Minimum inhibitory concentration (ppm)					
	Alternaria solani		*Botrytis cinerea*		*Fusarium solani*	
	MIC	MFC	MIC	MFC	MIC	MFC
Thyme	25	1000	50	1250	012/5	1000
Peppermint	50	1000	50	1500	50	1500
Khuzestani savory	25	1000	12/5	500	25	500
Mancozeb	10	50	20	*	20	*
Carbendazim	40	*	10	40	10	50

* No fungicidal effect was observed in the tested concentrations

fungicidal concentration (MFC) among the different concentrations of essential oils, showed that the lowest MFC is related to 500 µl/l savory essential oil on *B. cinerea* and *F. solani*. The lowest MFC of fungicides belonged to 40 µl/l carbendazim on *B. cinerea*. Mancozeb fungicide in tested concentrations could not control *B. cinerea* and *F. Solani* fungi well, also carbendazim fungicide in tested concentrations was not able to control *A. solani* well (Table 1).

Discussion بحث

Fungi causing post-harvest decay grow on a wide number of agricultural products and food by producing extracellular hydrolases, pectinases, proteinases, and amylases, cause their contamination (Bautista-Banos 2014). The use of plant essential oils in the control of plant pathogens has been proposed as a biological method in recent years and it has attracted the attention of many researchers as an effective method. Due to their antioxidant properties, these compounds increase the quality and storage length of fruits. (Anthony et al. 2003, El Ouadi et al. 2017;). The main characteristic of plant essential oils and their constituent compounds is their hydrophobicity. This feature enables the essential oils to penetrate into the lipids of the cytoplasmic membrane and the mitochondrial membrane of fungi and destroy the fungus cell (Cox et al. 2000). Tripathi et al. (2008) investigated the antifungal effects of the essential oils of 26 medicinal plants native to India on *B. cinerea* and concluded that

among them, the essential oils of 10 plants have a significant inhibition of mycelial growth compared to other essential oils, and by changing the concentration of the essential oils, the amount inhibition of mycelial growth changes. In a research, the effect of 39 medicinal plant essential oils on two storage fungi *B. cinerea* and *A. solani* and three soil fungi *Rhizoctonia solani*, *Fusarium oxysporum* and *Pythium ultimum*. was investigated. That the plant essential oils had different antifungal effects on the studied fungi. This difference can be related to the diversity and the ratio of the constituent compounds of herbal essential oils and their effect on the antifungal properties of essential oils (Ultee et al. 2002). Among the medicinal plants studied in this research, essential oil of thyme and savory have good antifungal effects, and peppermint has a weaker antifungal effect than these two essential oils. Bouchra et al. (2003) reported that thyme essential oil was more effective than peppermint in controlling *Botrytis cinerea*. It was also found that in all the examined essential oils, as the concentration of the essential oil increases, its antifungal effect also increases. Lee et al. (2007), showed that the antifungal activity of plant essential oils increases by increasing the concentration of essential oils.

The results of minimum inhibitory concentration (MIC) showed that among the different concentrations of the tested essential oils, the lowest MIC value corresponds to 12.5 ppm savory essential oil on *B. cinerea* and 12.5 µl/l thyme essential oil on *F. solani* or in other words, they had the most inhibitory effect on mycelium growth. The lowest MIC of the fungicides, or in other words, the highest growth inhibitory effect, was related to 10 µl/l mancozeb fungicide on *A. solani* and 10 µl/l carbendazim fungicide on *B. cinerea* and *F. solani*. The highest MIC or the lowest inhibitory rate belonged to 40 µl/l carbendazim fungicide on *A. solani*. These results were expected, Considering the antifungal effects observed in the essential oils and fungicides tested on the mentioned fungi. In a study conducted by Kumar et al. (2007), the fungicidal effect of thyme on eight *Alternaria* species, showed that this essential oil has a high inhibitory effect on different *Alternaria* species. In a research study on the effect of essential oils and extracts of 50 medicinal plants on *Candida albicans* fungus, it was shown that savory and thyme have stronger antifungal effects than mint (Naeini et al. 2011). The results of minimum fungicidal concentration (MFC) showed that among the different concentrations of essential oils, the lowest MFC or in other words, the highest fungicidal effect is related to 500 µl/l savory essential oil on *B. cinerea* and *F. solan*i and the highest amount MFC belonged to 1500 µl/l peppermint essential oil on *B. cinerea* and *F. solani* fungi. The lowest MFC rate of fungicides or the highest rate of fungicidal activity belonged to 40 µl/l carbendazim fungicide on *B. cinerea*. Mancozeb fungicide in tested concentrations could not control *B. cinerea* and *F. Solani* completely, and carbendazim fungicide in tested concentrations was not able to completely control *A. solani*. Feng et al. (2011), showed that thyme essential oil at a concentration of 1000 µl/l for 6 to 12 days completely inhibited the growth of *A. alternata*. In another study, the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) in thyme

plant essential oil were measured and compared with each other, and it was reported that thyme essential oil had strong fungicidal properties and inhibited the growth of *B. cinerea*.

Conclusion نتیجه‌گیری

In general, it can be concluded that among the essential oils tested in this study, low concentrations of the savory essential oil had very strong inhibitory and fungicidal effects on all three fungi, *A. solani*, *B. cinerea*, and *F. solani*, which this issue can justify the use of this essential oil in the management of storage diseases economically. Due to the adverse effects of chemical fungicides on human health and the environment, the above mentioned essential oils can be a suitable alternative for chemical fungicides to control storage fungi.

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